Reference: *Biol. Bull.*, **150**: 286–293. (April, 1976)

EFFECTS OF SALINITY AND TEMPERATURE ON THE DEVELOPMENT OF EGGS IN THE TUBE BUILDING AMPHIPOD *COROPHIUM TRIAENONYX* STEBBING

K. SHYAMASUNDARI

Department of Zoology, Andhra University, Waltair, South India

The amphipod *Corophium triaenonyx* is a fouling organism living in parchment tubes on timber and concrete jetties. It is a hardy form, tolerating wide ranges of salinity and temperature under laboratory conditions (Shyamasundari, 1973). It occurs abundantly throughout the year with peak numbers between November and March (Shyamasundari, 1972).

It is generally agreed that salinity and temperature play a major role in the development of embryos. The rate of embryonic development has been reported in several cases to be affected by salinity and the variation in salinity may modify the time and length of breeding season. Temperature fluctuations may enlarge or narrow the salinity range of the species and the salinity in turn may influence the temperature tolerance (Kinne, 1952; 1953a; 1956a; 1957; 1958a; and 1959). There have been quite a number of studies on reproduction and development in amphipods but contributions towards the effects of salinity and temperature or the combined effects of these parameters on development have been documented by a few papers. Most of the literature is based on field observations. Clemens (1950) studied the effect of salinity and temperature on the incubation period (time between fertilization and hatching) in *Gammarus fasciatus*. Bovie (1951) dealt with the pre-embryonic, post-embryonic and adult stages of *Hyalella azteca*. Kinne (1953a; 1959; 1960; 1961; and 1964) furnished information on the effects of salinity and temperature and their combined effects on the development of embryos, number of eggs and upper limiting temperature for embryonic development in *Gammarus duebeni*. Hynes (1954) showed the differences in the capacity of egg laying between freshwater and brackish-water forms of *Gammarus duebeni*. McLusky (1967; 1968) has given some details about the effects of salinity on survival, molting and growth activities of *Corophium volutator*. Vlasblom and Bolier (1971) studied the tolerance of embryos of *Marinogammarus marinus* and *Orchestia gammarella* to low salinities. The duration of embryonic development at various temperatures in *Gammarus duebeni, Gammarus lawrencianus, Gammarus obtusatus, Gammarus occanicus* and *Gammarus setosus* was studied by Steele and Steele (1973). Dorgelo (1974) discussed the effect of salinity and temperature combinations on *Chaetogammarus marinus, Gammarus tigrinus* and *Gammarus forsarum* from marine, freshwater and brackish-water, respectively. Shyamasundari and Hanumantha Rao (1974) furnished some details about the effects of temperature on the embryonic and post-embryonic stages of *Corophium triaenonyx*.

An experimental study has been undertaken in order to ascertain more precisely the effects of salinity and the combined effects of salinity and temperature on the development of eggs in *C. triaenonyx*.

Materials and Methods

Specimens of *C. triaenonyx*, after collection from jetties in the Visakhapatnam Harbor, were transferred to stock tanks. Berried females were separated and maintained in large tanks to obtain fertilized eggs. All the eggs in a single brood were in the same stage of development. Removal of eggs from the brood pouch with a sterile needle was done according to the method followed by Shyamasundari and Hanumantha Rao (1974). Usually eggs at 64 cell stage were used for all experiments. Fifty eggs were used in each experiment.

To each of the fingerbowls was added 50 cc of sea water of known salinity. A series of salinities covering a range from 7.5‰ to 37.5‰ at 2.5‰ intervals was prepared. Different concentrations were prepared as described by Shyamasundari

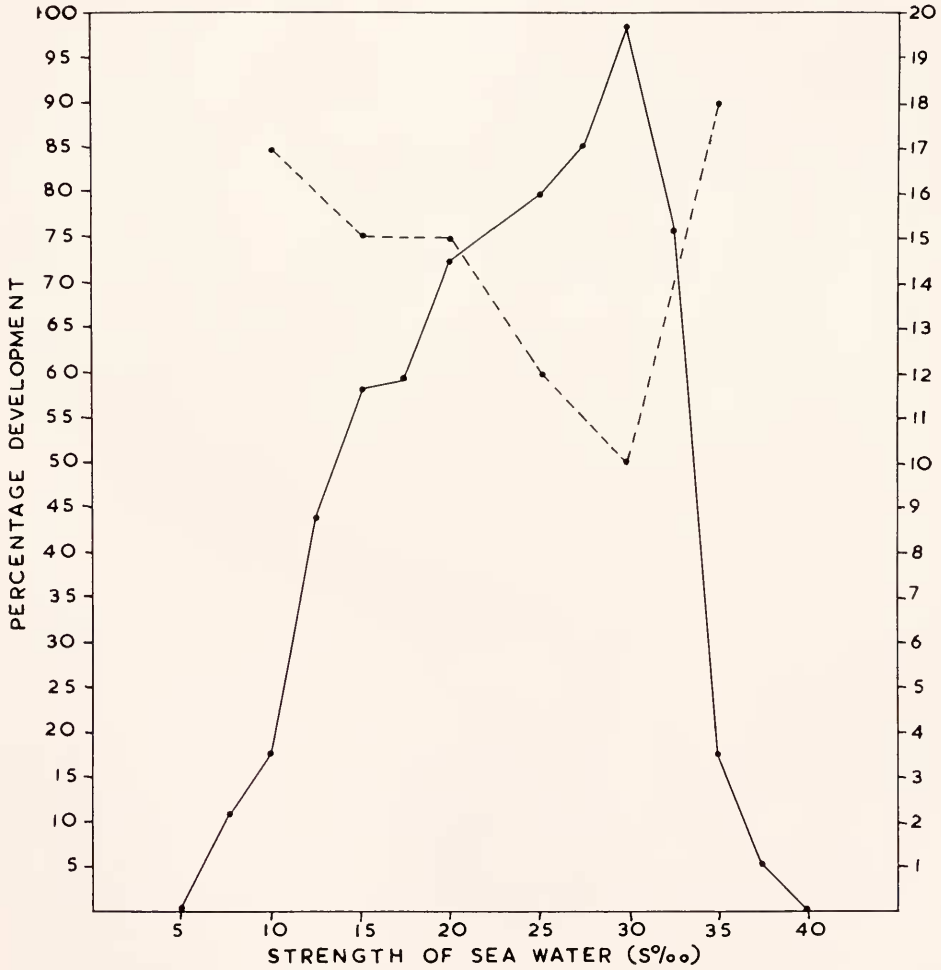

Figure 1. Effect of salinity on the percentage development and incubation period of eggs of *Corophium triaenonyx* Stebbing. The temperature was held constant at 28° C (±0.1° C).

(1973). Each experiment was repeated three times. All cultures were maintained at a constant temperature of 28° C (± 0.1° C). The dishes were examined daily and the degenerating eggs were removed. Water was changed every day.

To test the combined effect of salinity and temperature, six salinities (10‰, 15‰, 20‰, 25‰, 30‰, and 35‰) and five temperatures (9° C, 15° C, 22° C, 28° C, and 35° C) were chosen. Duplicate cultures were established at each of the six salinities and five temperatures tested, giving a total of 30 combinations and 60 cultures in each experiment. For all experiments controls were maintained. Normally, development in amphipods is completed in ten days time at salinity 30‰ and temperature 28° C. For convenience the entire development was divided into three stages: Stage I, advanced gastrula stage; Stage II, formation of rudimentary appendages; and Stage III, formation of eye.

Results

Corophium triaenonyx eggs developed into young ones throughout a wide range of salinities from 7.5‰ to 37.5‰ at 28° C (± 0.1° C) maintained in the laboratory. At a higher concentration of 40‰ and a lower concentration of 5‰ development ceased. At 37.5‰ only 5.3% of the embryos developed into normal young ones and at 35‰, 17.5% developed normally. At 7.5‰ and 10‰ only 10.5% and 17.5%, respectively, of the embryos developed normally. Approximately 44.5% of the embryos developed into young ones at 12.5‰ but the salinity range for a satisfactory development of at least 58.5% was between 15‰ and 17.5‰. Similarly more than 70% of the embryos developed into young ones between 20‰ and 32.5‰, but the salinity range for a very high percentage (80%–95%) was between 27.5‰ and 30‰. With temperature constant at 28° C (± 0.1° C), the optimum salinity range for embryonic development is 27.5‰ to 30‰. The results are presented graphically in Figure 1.

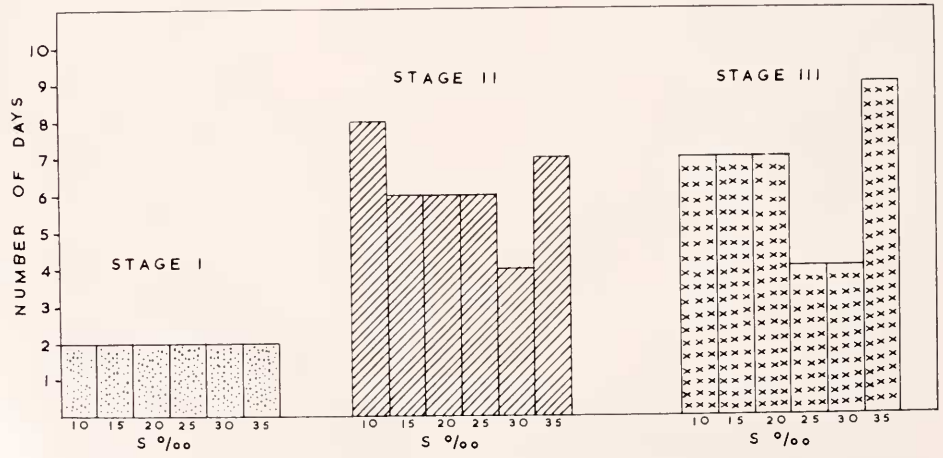

Figure 2. Effect of salinity on various stages of development of eggs of *Corophium triaenonyx* Stebbing. The temperature was held constant at 28° C (±0.1° C).

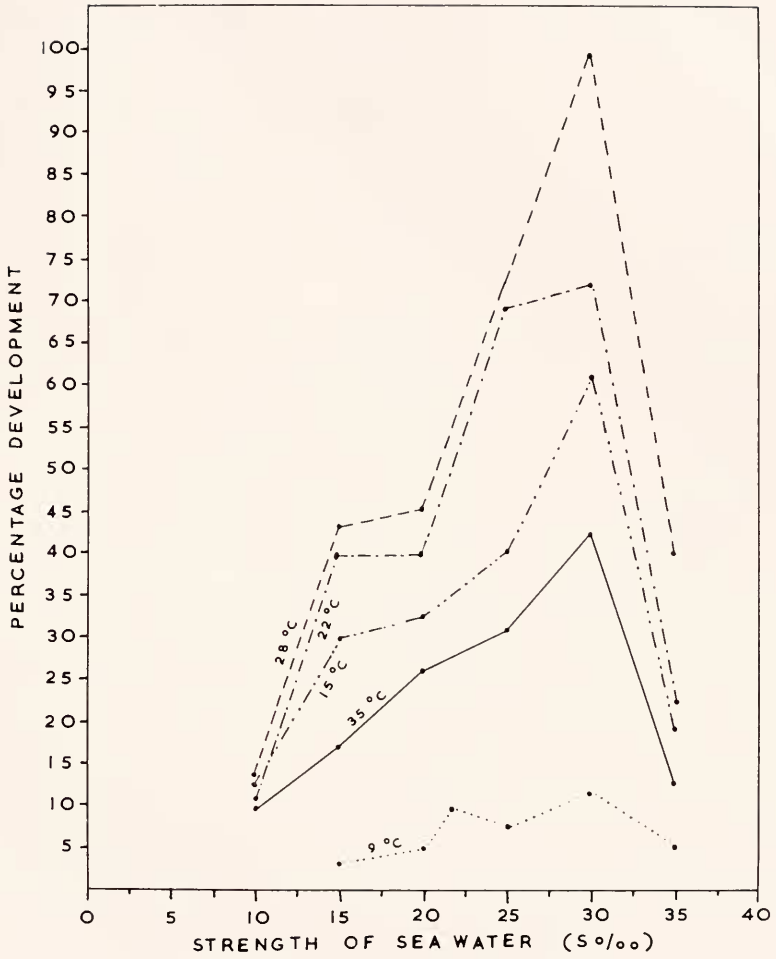

FIGURE 3. Combined effect of salinity and temperature on the percentage development of eggs of *Corophium triaenonyx* Stebbing.

The overall time required for complete and successful development was prolonged at lower and higher salinities (35‰ and 10‰). At 35‰ only 17.5% developed normally, and it was completed in 18 days time; at 30‰, in 10 days; at 25‰ it was spread over 12 days; it was 15 days at 15‰–20‰; and 17 days at 10‰. The results are given graphically in Figure 1.

At 35‰ completion of the first stage required only 2 days, second stage 7 days and third 9 days. At 30‰ the first stage required 2 days, second and third stages required 4 days each. For the eggs kept at 25‰ the first stage required 2 days, second stage 6 days and third 4 days. Development at 20‰ and 15‰ was almost similar. The first stage required 2 days, second stage 6 days and third 7 days. At the lowest salinity tested only 17.5% developed into young

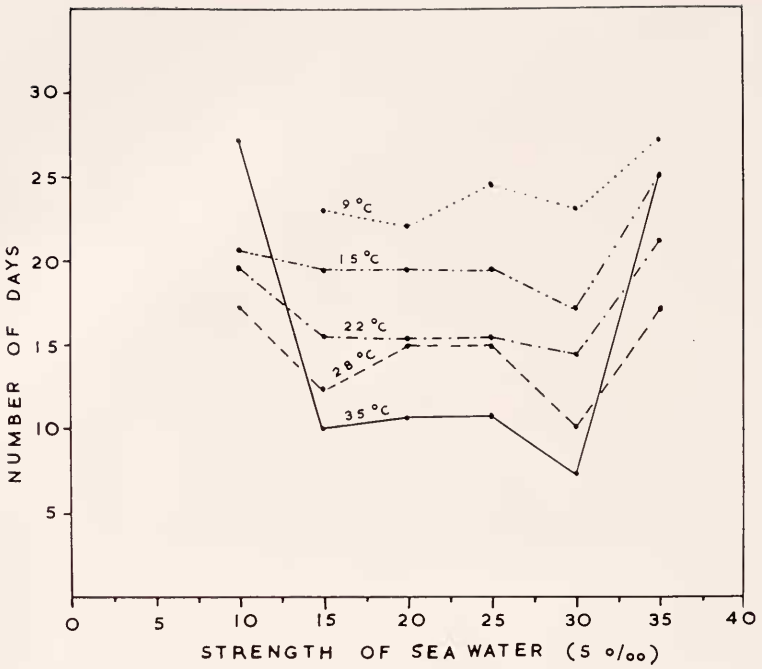

FIGURE 4. Combined effect of salinity and temperature on the incubation period of eggs of *Corophium triaenonyx* Stebbing.

ones. The first stage required 2 days, second stage 8 days and third 7 days. Results are presented graphically in Figure 2.

Observations on the combined effects of salinity and temperature revealed that 70% of the eggs developed into young ones with the dashlined boundary which was circumscribed by temperatures from 22° C to 28° C and salinity from 25‰ to 30‰. 99% was obtained at a temperature of 28° C and salinity 30‰ but this percentage was drastically reduced to 10%–13% at 10‰. At the lowest temperature tested (9° C), the percentage of development at all salinities was drastically reduced. The borderline appears to be 15° C in that a normal number of eggs (30%–40%) developed at 15‰, 20‰, and 25‰; 60% developed at 30‰; and 19.5%, at 35‰. Though development was hastened at 35° C between 15‰ and 30‰, at no salinity tested did 50% of the eggs develop into young ones. At 35‰ at none of the temperatures tested did as many as 50% of the eggs develop. Only a maximum of 40% was obtained at 28° C but this salinity was well above optimum and almost approached the upper limit of the embryonic development of *C. triaenonyx*. Results are given graphically in Figure 3.

At 9° C and 10‰ development ceased. A more detailed analysis of the data revealed that there was not much difference at 15° C and 22° C between 15‰ and 25‰. Development at 30‰ at all temperatures tested was dependent on temperature; that is, with an increase in temeprature the incubation period was reduced. At extreme salinities (10‰ and 35‰) the development was retarded

at higher temperatures of 35° C. At 9° C at all salinities tested it was more than double the normal time. At 35° C between 15‰ and 30‰ the development time was between 7–11 days; but at 10‰ and 35‰, it was spread over 27 and 25 days, respectively. Results are presented graphically in Figure 4.

Two important points were noticed during the course of the experiments. First, the effect of salinity on various stages seems to be different. In all concentrations tested there was no variation in the time taken for the first stage (advanced gastrula stage) to be completed. Secondly, there is a relationship between the poor percentage of development and prolonged incubation periods.

Discussion

The salinity tolerance in amphipods appeared to be different in different stages. Eggs of *Corophium triaenonyx* are found to develop at a fairly wide range of salinity, from 7.5‰ to 37.5‰, although the adults are able to tolerate a range between 0.6‰ to 59.8‰. Kinne (1953a) states that the eggs of *Gammarus duebeni* (near Kiel, Germany) can not develop below 1‰, though the adults live indefinitely in fresh water. The eggs of the shore crab *Carcinus maenas* develop normally only in salinities between 28‰ to 40‰, while adults can tolerate salinities below 4‰ (Broekhuysen, 1936). Similar findings have been reported in *Balanus* by Barnes (1953). It can be seen that although the eggs of *C. triaenonyx* developed through the above mentioned range, satisfactory percentage (70%) was obtained at a slightly smaller range of 20‰–32.5‰ and a maximum percentage at a still smaller range of 27.5‰–30‰. This was considered as the optimum range at a constant temperature of 28° C. According to Crisp and Costlow (1963) in *Balanus eburneus, Balanus amphitrite amphitrite* and *Chelonobia patula,* development took place at a normal rate at 25‰ to 40‰; but when they were exposed to salinities of 25‰–15‰ or 40‰–60‰, only a portion of the eggs hatched.

The normal development rate was 10–12 days between 25‰–30‰, but it was greatly prolonged (17–18 days) at extreme salinities (10‰ and 35‰). It is apparent from the results that extremes of salinities impose more limitations on development. Extreme salinities reduce the reproductive performance (Kinne, 1956b, 1956c, and 1958b). In England *Gammarus duebeni* of brackish-water lays more eggs than its counterpart in fresh water (Hynes, 1954). The optimal range of salinity for successful development in *Gammarus duebeni* seems to be 2‰–10‰ at a temperature of 8° C (Kinne, 1959). Kinne (1953a) reported that *Gammarus duebeni*, for which incubation period was determined at various salinities at 18° C–20° C, produced an average of 18 eggs in 2‰, 27 eggs at 10‰ and 17 eggs at 30‰. Vlasblom and Bolier (1971) stated that the incubation rate remained unaltered by changes in salinities but the juvenile number decreased.

Development ceases at 9° C at lower salinity of 10‰. The maximum percentage of development was noticed in salinities of 25‰–30‰ at temperatures 22° C–28° C. Fairly satisfactory development was observed at 20‰ and 15‰ at all temperatures. The incubation period was retarded at 35‰ and 10‰. According to Kinne (1953a) in *Gammarus duebeni* at 5° C and 6° C there was no significant difference, but above 8° C a progressive retardation was noticed both in 2‰ and 30‰ relative to the values observed at 10‰.

The lower limiting salinity for development in *C. triaenonyx* is 15‰ at 9° C and 10‰ between 15° C–35° C. According to Broekhuysen (1936) the lower limiting salinity for development of *Carcinus maenas* is about 20‰ at 16.3° C and 26‰ at 10° C. A close relationship between the effect of salinity on the rate of development and survival has been demonstrated by Costlow, Bookhout and Monroe (1960) in several brachyuran larvae.

It is clear that the distribution of *Corophium triaenonyx in* Visakhapatnam Harbor, and presumably in other brackish and estuarine habitats, will be limited by the effects of salinity and the combined effects of salinity and temperature. It should be possible to define in terms of the physical properties of the waters (ranges of salinity in relation to ranges of temperatures) which zones of harbors and estuaries will support stocks of *C. triaenonyx* capable of successful reproduction and therefore where these tube building amphipods are likely to become established as permanent elements of the local fauna.

I am grateful to Professor K. Hanumantha Rao, Head of the Department of Zoology, Andhra University for guidance and helpful criticism.

Summary

1. Experiments were conducted to determine the percentage of development and incubation rate of eggs of *Corophium triaenonyx* at various salinities from 5‰ to 40‰.

2. The eggs develop only through a range of salinities from 7.5‰ to 37.5‰, although the adults are able to tolerate a range bebtween 0.6‰ to 59.8‰. A satisfactory percentage of development occurred between 20‰ and 32.5‰, but the optimum range was between 27.5‰ and 30‰ at a constant temperature of 28° C (± 0.1° C). Extreme salinities retarded the incubation period, being 18 and 17 days at 35‰ and 10‰, respectively.

3. In the experiments conducted to determine the combined effect of salinity and temperature, it was revealed that the best results were obtained at a combination of 30‰ and 28° C. The upper limiting salinity appeared to be 35‰ at all temperatures and lower limiting salinity is 10‰ between 15°C and 35° C and 15‰ at 9° C. High temperatures at extreme salinities retarded the development.

LITERATURE CITED

Barnes, H., 1953. The effect of lowered salinity on some barnacle nauplii. *J. Anim. Ecol.*, **22**: 328–330.

Bovie, E. C., 1951. Some effects of temperature on the rates of embryonic, pre-embroynic and adult growth in *Hyalella azteca* Sassure. *Proc. Iowa. Acad. Sci.*, **59**: 439–444.

Broekhuysen, G. J., 1936. On development, growth and distribution of *Carcinus maenas* L. *Arch. Neerland. Zool.*, **2**: 257–399.

Clemens, H. P., 1950. Life cycle and ecology of *Gammarus fasciatus* Say. *Contrib. Stone Lab. Ohio. Univ.*, **12**: 1–63.

Costlow, J. D., C. G. Bookhout, and R. Monroe, 1960. The effect of salinity and temperature on larval development of *Sesarma cinereum* (Bosc) reared in the laboratory. *Biol. Bull.*, **118**: 183–202.

Crisp, D. J., and J. D. Costlow, 1963. The tolerance of developing cirripede embryos to salinity and temperature. *Oikos*, **14**: 22–34.

Dorgelo, J., 1974. Comparative ecophysiology of gammarids (Crustacea:Amphipoda) from marine, brackish and fresh water habitats exposed to the influence of salinity temperature combinations. I. Effect on survival. *Hydrobiol. Bull. (Amsterdam)*, **8**: 90–108.

Hynes, H. B. N., 1954. The reproductive cycle of some British fresh water gammaridae. *J. Anim. Ecol.*, **24**: 352–387.

Kinne, O., 1952. Zur Biologie und Physiologie von *Gammarus duebeni* Lillj V: Untersuchungen Über Blutkonzentration, Herzfrequenz und Atmung. *Kieler Meeresforsch.*, **9**: 134–150.

Kinne, O., 1953a. Zur Biologie und Physiologie von *Gammarus duebeni* Lillj I: *Z. Wiss. Zool.*, **157**: 427–491.

Kinne, O., 1953b. Zur Biologie und Physiologie von *Gammarus duebeni* Lillj. II: Über die Häutungsfrequenz, ihre Abhängigkeit von Temperatur und Salzgehalt sowie über ihr Verhalten bei isoliert gehaltenen und amputierten Versuchstieren. *Zool. Jahrb. Abt. Allg. Zool. Physiol. Tiere*, **4**: 183–206.

Kinne, O., 1956a. Über Temperatur und Salzgehalt und ihre Physiologisch-biologische Bedeutung. *Biol. Zbl.*, **75**: 314–327.

Kinne, O., 1956b. Uber den Einfluβ der Salzgehaltes und der Temperatur auf Wachtum, Form und Vermehrung bei dem Hydroidpolypen *Cordylophora caspia* (Pallas). Thecata: Clavidae. *Zool. Jahrb. Abt. Zool. Physiol. Tiere*, **66**: 565–638.

Kinne, O., 1956c. Zur Ökologie der Hydroidpolypen der Nordostseekanals. *Z. Morph. Tiere*, **45**: 217–249.

Kinne, O. 1957. A programatic study of comparative biology of marine and brackish water animals. *Ann. Biol.* **33**: 87–92.

Kinne, O., 1958a. Über Temperatur und Salzgehalt und ihre Physiologisch-biologisch Bedeutung. *Biol. Zbl.*, **75**: 314–327.

Kinne, O., 1958b. Über die Reaktion erbgleichem Coelentuatengewebes auf Verschiedene Saltgehalts und Temperaturbendingungen. *Zool. Jahrb. Abt. Allg. Zool. Physiol. Tiere*, **67**: 407–486.

Kinne, O., 1959. Ecological data on the amphipod *Gammarus duebeni* Lillj. A Monograph. *Veroff. Inst. Meeresforsch. Bremerhaven*, **6**: 177–202.

Kinne, O., 1960. *Gammarus salinus*—einige Daten über den Unwelteinfluβ auf Wachstum, Hautungsfolge Herzfrequenz und Eientwicklungsdauer. *Crustaccana.*, **1**: 208–217.

Kinne, O., 1961. Growth, moulting frequency, heart beat, number of eggs and incubation time in *Gammarus zaddachi* exposed to different environments. *Crustaccana.*, **2**: 26–36.

Kinne, O., 1964. The effects of temperature and salinity on marine and brackish water animals. 2. Salinity and temperature-salinity combinations. *Oceanogr. Mar. Biol. Annu. Rev.*, **2**: 281–339.

McLusky, D. S., 1967. Some effects of salinity on the survival, moulting and growth of *Corophium volutator* (Amphipoda). *J. Mar. Biol. Ass. U.K.*, **47**: 607–617.

McLusky, D. S., 1968. Some effects of salinity on the distribution and abundance of *Corophium volutator* in Ythan estuary. *J. Mar. Biol. Ass. U.K.*, **48**: 443–454.

Shyamasundari, K., 1972. Studies on the tube building amphipod *Corophium triaenonyx* Stebbing from Visakhapatnam harbour: Annual life cycle. *Riv. Biol.*, **25**: 503–516.

Shyamasundari, K., 1973. Studies on the tube building amphipod *Corophium triaenonyx* Stebbing from Visakhapatnam harbor: Salinity and temperature tolerance. *Biol. Bull.*, **144**: 503–510.

Shyamasundari, K., and K. Hanumantha Rao, 1974. Effect of temperature on embryonic and post-embryonic stages of *Corophium triaenonyx* Stebbing (Crustacea:Amphipoda). *Broteria*, **70**: 193–200.

Steele, D. H., and V. J. Steele, 1973. The biology of *Gammarus* (Crustacea:Amphipoda) in the North Western Atlantic VII. The duration of embryonic development in five species at various temperatures. *Can. J. Zool.*, **51**: 995–999.

Vlasblom, A. G., and G. Bolier, 1971. Tolerance of embryos of *Marinogammarus marinus* and *Orchestia gammarella* (Amphipoda) to lowered salinities. *Netherlands J. Sea. Res.*, **5**: 334–341.